Dkt. 2259/65532-A-PCT/JPW/AJM/MVM

IN THE UNITED STATES RECEIVING OFFICE (RO/US)

Applicant	:	Roger Williams Hospital
International Application No.:		PCT/US03/12679
International Filing Date	:	April 23, 2003
U.S. Serial No.:		10/553,853
For	:	COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

1185 Avenue of the Americas
New York, New York 10036
February 28, 2006

Attn: PCT Legal Office
Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RENEWED PETITION FOR REVIVAL OF AN INTERNATIONAL APPLICATION FOR PATENT DESIGNATING THE U.S. ABANDONED UNINTENTIONALLY UNDER 37 C.F.R. §1.137(b)

This Renewed Petition is submitted to revive the above-identified PCT International Application ("subject application") under 37 C.F.R. §1.137(b). Petitioner hereby requests reconsideration, pursuant to 37 C.F.R. §1.137(e), of a December 30, 2005 Decision on petitioner's October 19, 2005 Petition, which Decision was issued by the Office of PCT Legal Administration at the United States Patent and Trademark Office ("USPTO") in connection with the subject application. 37 C.F.R. §1.137(e) provides a period of two months for filing a request for reconsideration. Therefore, a request is due by February 28, 2006. Accordingly, this Renewed Petition is being timely filed.

On October 19, 2005, petitioner submitted a Petition For Revival Of An International Application For Patent Designating the U.S.

Abandoned Unintentionally Under 37 C.F.R. §1.137(b) in connection with the subject application. A copy of the October 19, 2005 Petition, with Exhibits, is annexed hereto as **Exhibit 1**.

The October 19, 2005 Petition set forth certain facts relating to the subject application and the events preceding and following its abandonment. In part, the October 19, 2005 Petition stated the following: (i) the thirty (30) month deadline for entering the national stage in the United States was October 23, 2004; (ii) Kimberly O'Connell, Esq., Vice President and General Counsel of petitioner Roger Williams Hospital ("Roger Williams"), was advised of the October 23, 2004 deadline by John P. White, Esq. of Cooper & Dunham LLP ("Cooper & Dunham"), patent counsel for Roger Williams, as late as October 7, 2004; (iii) through oversight, Ms. O'Connell did not instruct Cooper & Dunham to enter the national stage in the United States by that deadline; (iv) prior to October 5, 2005, Ms. O'Connell communicated on several occasions with Alan J. Morrison, Esq., the undersigned attorney, regarding, among other things, the possibility of entering the national stage in the United States; and (v) on October 5, 2005, Ms. O'Connell first informed Mr. Morrison that her not instructing Cooper & Dunham to enter the national stage in the United States was due to her oversight and was thus unintentional.

In the December 30, 2005 Decision, Ms. Cynthia Kratz, Attorney Advisor with the Office of PCT Legal Administration, acknowledged receipt of (i) the transmittal papers for entry into the national stage in the U.S., including the national stage filing fee, and (ii) the petition fee, pursuant to 37 C.F.R. §1.137(b), all contained in the October 19, 2005 Petition.

However, Ms. Kratz stated in the Decision that the October 19, 2005 Petition is dismissed because petitioner allegedly did not provide a showing to the satisfaction of the Commissioner that the entire delay in filing the required reply from the due date for the reply until the filing of a grantable petition pursuant to 37 C.F.R. §1.137(b) was unintentional. Therefore, Ms. Kratz stated in the Decision that the application remains abandoned.

Specifically, Ms. Kratz further stated that "the fact that communications between applicant and counsel were held regarding the continued interest in pursuing national stage prior to October 5, 200[5] without filing the national stage papers earlier raises the question as to whether the delay was unintentional." Ms. Kratz stated that "[p]etitioner should provide a statement explaining the communications between counsel and petitioner 'prior to October 5, 2005' showing that the delay in filing the national stage application was unintentional."

In response, petitioner attaches hereto as **Exhibit 2** a copy of a Declaration In Support Of Renewed Petition Under 37 C.F.R. §1.137(b) signed by Ms. Kimberly O'Connell. In the Declaration, Ms. O'Connell declares, in part, the following:

i. "John P. White, Esq. of Cooper & Dunham LLP ('Cooper & Dunham'), patent counsel for Roger Williams Hospital, notified me in writing of the October 23, 2004 deadline (i.e., 30 months from the April 23, 2002 priority date) for entering the national stage in the United States for the subject application."

ii. "Prior to October 23, 2004, it was my intention to enter the national stage for the subject application

in the United States. However, through my oversight, I lost track of the October 23, 2004 deadline for entering the national stage in the United States and therefore did not timely instruct Cooper & Dunham to do so. Accordingly, Cooper & Dunham did not timely enter the national stage in the United States with respect to the subject application."

iii. "From October 23, 2004 until the present, it was never my intent not to enter the national stage in the United States."

iv. "Again, through my oversight, I did not act in connection with the subject application from October 23, 2004 until late June, 2005, when Alan J. Morrison, Esq. of Cooper & Dunham, after receiving a telephone inquiry in late June, 2005 from Dr. Lawrence Lum who is a coinventor named on the subject application, caused me to focus on the fact that the subject application never entered the national stage in the United States."

v. "On June 27, 2005, I contacted Mr. Morrison and inquired whether any steps could still be taken to enter the national stage in the United States."

vi. "Between June 27, 2005 and October 5, 2005, I communicated via telephone and e-mail on several occasions with Mr. Morrison concerning the possibility of a late entry into the national stage in the United States. During these communications, Mr. Morrison discussed difficulties associated with late entry into

the national stage. During these communications, I did not inform Mr. Morrison that (i) my failure to provide instructions to Cooper & Dunham by October 23, 2004 to enter the national stage in the United States was due to an oversight and was unintentional, and (ii) my not focusing on this matter prior to June 27, 2005 was likewise an oversight and unintentional."

vii. "During an October 5, 2005 telephone discussion with, *inter alia*, Mr. Morrison, I first informed Mr. Morrison that (i) my failure to instruct Cooper & Dunham by October 23, 2004 to enter the national stage in the United States was due to my oversight and was unintentional, and (ii) my not focusing on this matter prior to June 27, 2005 was likewise an oversight and unintentional."

In addition to the facts declared by Ms. O'Connell in the attached Declaration, I, Alan J. Morrison, the undersigned attorney, hereby declare the following:

i. Neither I nor, to my knowledge, anyone else at Cooper & Dunham ever received instructions from Ms. O'Connell on or before October 23, 2004 to enter the national stage in the United States for the subject application. Accordingly, Cooper & Dunham never entered the national stage in the United States by the October 23, 2004 deadline for doing so.

ii. After October 23, 2004 and prior to late June, 2005, nothing had prompted me or, to my knowledge, anyone else at Cooper & Dunham to communicate with Ms.

O'Connell regarding the subject application and its status.

iii. Prior to an October 5, 2005 telephone discussion with, *inter alia*, Ms. O'Connell, I was not aware that (a) Ms. O'Connell's failure to instruct Cooper & Dunham by October 23, 2004 to enter the national stage in the United States was due to her oversight and was unintentional, and (b) Ms. O'Connell's not focusing on this matter prior to June 27, 2005 was likewise an oversight and unintentional. Once I became aware on October 5, 2005 of the unintentional nature of the subject application's abandonment, I promptly took steps to prepare the Petition under 37 C.F.R. §1.137(b) which was filed on October 19, 2005.

Finally, petitioner notes that on page 2 of the Decision, Ms. Kratz states that "[i]t is appropriate for the Office to require further information as to how the delay in discovering the abandoned status occurred despite the exercise of due care and diligence on the part of applicant and applicant's representatives." In a February 27, 2006 telephone message from Ms. Kratz to the undersigned attorney, Ms. Kratz stated that this requirement is based on M.P.E.P. §711.03(c).

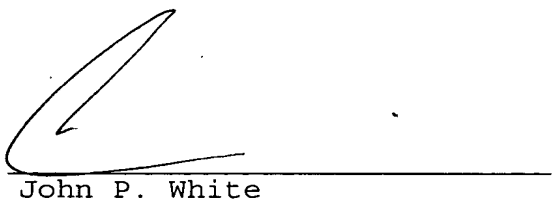
Petitioner maintains, however, that the provision of M.P.E.P. §711.03(c) presumably relied on by Ms. Kratz is not applicable to the October 19, 2005 Petition. Specifically, M.P.E.P. §711.03(c) provides, in relevant part, that "[w]here a petition pursuant to 37 C.F.R. §1.137(a) or (b) is not filed *within one year of the date of abandonment of the application* . . . the Office will require: . . . a showing as to how the delay in discovering the

abandoned status of the application occurred despite the exercise of due care or diligence on the part of the applicant (or applicant's representative)." (emphasis added) In this case, the October 19, 2005 Petition was filed *less than one year* after the October 23, 2004 abandonment of the subject application. Petitioner therefore need not provide a showing as to how the delay in discovering the abandoned status of the application occurred despite the exercise of due care or diligenceon the part of the applicant or applicant's representative.

In view of the above facts and remarks, petitioner respectfully requests that (a) this Renewed Petition be granted, (b) the subject application be revived, and (c) the United States national stage application submitted to the USPTO on October 19, 2005 be accepted.

No fee is deemed necessary in connection with the filing of this Renewed Petition. If any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Petitioner
Cooper & Dunham, LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

Exhibit 1

Dkt. 2259/6553 PCT/JPW/AJM/MVM

IN THE UNITED STATES RECEIVING OFFICE (RO/US)

Applicant : Roger Williams Hospital

Intl Application No.: PCT/US03/12679

Intl Filing Date : April 23, 2003

For : COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

1185 Avenue of the Americas
New York, New York 10036
October 19, 2005

Mail Stop PCT
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTN: PCT Legal Staff

Sir:

EXPRESS MAIL CERTIFICATE OF MAILING FOR ABOVE-IDENTIFIED APPLICATION

"Express Mail" mailing label number: EV 553 659 986 US
Date of Deposit: October 19, 2005
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, ATTN: PCT Legal Staff.

Printed Name:

Respectfully submitted,

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicant
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

Applicant: Roger Williams Hospital
Intl Appln No.: PCT/US03/12679
Intl Filing Date: April 23, 2003
Exhibit 1

Dkt. 2259/65532-A-PCT/JPW/AJM/MVM

IN THE UNITED STATES RECEIVING OFFICE (RO/US)

Applicant : Roger Williams Hospital

International Application No.: PCT/US03/12679

International Filing Date : April 23, 2003

For : COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

1185 Avenue of the Americas
New York, New York 10036
October 19, 2005

Attn: PCT Legal Staff
Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PETITION FOR REVIVAL OF AN INTERNATIONAL APPLICATION FOR PATENT DESIGNATING THE U.S. ABANDONED UNINTENTIONALLY UNDER 37 C.F.R. §1.137(b)

This Petition is submitted to revive the above-identified application under 37 C.F.R. §1.137(b).

Requirements of Petition to Revive

A petition under 37 C.F.R. §1.137(b) must be accompanied by:

(1) the required reply, unless it has been previously filed;

(2) the petition fee set forth in 37 C.F.R. §1.17(m);

(3) a statement that the entire delay in filing the required reply from the due date for the reply until

the filing of a grantable petition pursuant to this paragraph was unintentional; and

(4) any terminal disclaimer (and fee set forth in §120(d)) required pursuant to 37 C.F.R. §1.137(c) for a utility application filed before June 8, 1995.

Statement of Facts

The thirty (30) month deadline for entering the national stage in the United States Patent and Trademark Office for PCT International Application No. PCT/US03/12679 (subject application) was October 23, 2004. Kimberly O'Connell, Esq., Vice President and General Counsel of applicant Roger Williams Hospital (Roger Williams), was advised of the October 23, 2004 deadline by John P. White, Esq. of Cooper & Dunham LLP, patent counsel for Roger Williams, as late as October 7, 2004. However, through oversight, Ms. O'Connell did not instruct Cooper & Dunham LLP to enter the national stage in the United States by that deadline.

Prior to October 5, 2005, Ms. O'Connell communicated on several occasions with Alan J. Morrison, Esq., the undersigned attorney, regarding, among other things, applicant's continued interest in entering the national stage in the United States for the subject application and whether doing so would be possible. On October 5, 2005, Ms. O'Connell first informed Mr. Morrison that her not instructing Cooper & Dunham LLP to enter the national stage in the United States was due to her own oversight and was thus unintentional.

Required Reply Under 37 C.F.R. §1.137(b)(1)

The above-identified application became abandoned as to the United States because the fees and documents required by 35 U.S.C. §371(c) were not filed prior to the expiration of the time set in 37 C.F.R. 1.495(b).

Pursuant to 35 U.S.C. §371(c):

> "The applicant shall file in the Patent and Trademark Office -
>
> (1) the national fee provided in section 41(a) of this title;
>
> (2) a copy of the international application, unless not required under subsection (a) of this section or already communicated by the International Bureau, and a translation into the English language of the international application, if it was filed in another language;
>
> (3) amendments, if any, to the claims in the international application, made under article 19 of the treaty, unless such amendments have been communicated to the Patent and Trademark Office by the International Bureau, and a translation into the English language if such amendments were made in another language;

(4) an oath or declaration of the inventor (or other person authorized under chapter 11 of this title) complying with the requirements of section 115 of this title and with regulations prescribed for oaths or declarations of applicants; and

(5) a translation into the English language of any annexes to the international preliminary examination report, if such annexes were made in another language."

Applicants attach hereto as **Exhibit 1** the necessary papers and the fee for filing a national stage application pursuant to 35 U.S.C. §371, i.e. specification (41 pages), claims (12 pages), Preliminary Amendment including a new Abstract of the Disclosure (Exhibit A), Declaration and Power of Attorney (unsigned), Transmittal Letter (in duplicate), a check in the amount of $800.00, and an Express Mail Certificate of Mailing bearing Label No. EV 553 659 986 US dated October 19, 2005. Accordingly, the required reply is being submitted.

Petition Fee Required Under 37 C.F.R. §1.137(b)(2)

The required fee for filing a Petition under 37 C.F.R. §1.137(b) as set forth in 37 C.F.R. §1.17(m) is $750.00 for a small entity and applicants enclose a check for this amount.

Statement Under 37 C.F.R. §1.137(b)(3)

The entire delay in filing the required reply, i.e. the attached application for entering the national stage in the United States

under 35 U.S.C. §371, from the due date for filing the application until the filing of this petition pursuant to 37 C.F.R. §1.137(b), was unintentional.

Terminal Disclaimer Under 37 C.F.R. §1.137(b)(4)

Because the subject application was filed on April 23, 2003, which is after June 8, 1995, 37 C.F.R. §1.137(c) does not require any terminal disclaimer to be filed.

In view of the foregoing, applicants earnestly solicit an expeditious revival of the subject application.

If a telephone interview would be of assistance in resolving any issue in connection with this petition, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the enclosed $750.00 petition fee as set forth in 37 C.F.R. §1.17(m) and the enclosed $800.00 filing fee as set forth in 37 C.F.R. §1.492, is deemed necessary in connection with the filing of this petition. If any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicant
Cooper & Dunham, LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

Exhibit 1

PTO-1390 (Rev. 07-2005)
ed for use through 3/31/2007. OMB 0651-0021
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER: 65532-A-PCT-US/JPW/JW U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US2003/012679	April 23, 2003	April 23, 2002

TITLE OF INVENTION
COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

APPLICANT(S) FOR DO/EO/US
Lawrence G. Lum and Randall J. Lee

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. [x] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [x] The US has been elected (Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [x] is attached hereto (required only if not communicated by the International Bureau). Specification .....41pp Claims 12pp
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [x] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [x] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). **(unsigned)**
10. [ ] An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A preliminary amendment.
14. [ ] An Application Data Sheet under 37 CFR 1.76.
15. [ ] A substitute specification.
16. [ ] A power of attorney and/or change of address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.2 and 37 CFR 1.821- 1.825.
18. [ ] A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 15 minutes to complete, including gathering information, preparing, and submitting the completed form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PTO-1390 (Rev. 07-2005)
Approved for use through 3/31/2007. OMB 0651-0021
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/US2003/012679	65532-A-PCT-US/JPW/JW

20. Other items or information: **Return receipt postcard; Express Mail Certificate of Mailing dated October 19, 2005, bearing Express Mail Label No. EV 553659986 US**

The following fees have been submitted	CALCULATIONS	PTO USE ONLY
21. ☒ Basic national fee (37 CFR 1.492(a)) $300	$ 300	
22. ☒ Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... $0 All other situations $200	$ 200	
23. ☒ Search fee (37 CFR 1.492(b)) If the written opinion of the ISA/US or the International preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... $0 Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority ..... $100 International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB ..... $400 All other situations $500	$ 100	
TOTAL OF 21, 22 and 23 =	600	
☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) or computer program listing in an electronic medium) (37 CFR 1.492(j)). The fee is **$250** for each additional 50 sheets of paper or fraction thereof.		

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round up to a whole number)	RATE	
53 - 100 =	0 /50 =	0	x $250	$ 0

Surcharge of **$130.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	19 - 20 =	0	x $ 50	$ 0
Independent claims	8 - 3 =	5	x $200	$ 1000
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $360	$ 0
			TOTAL OF ABOVE CALCULATIONS =	$ 1600
☒ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by ½.				
			SUBTOTAL =	$ 800
Processing fee of **$130.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$
			TOTAL NATIONAL FEE =	$ 800
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
			TOTAL FEES ENCLOSED =	$ 800
			Amount to be refunded:	$
			Amount to be charged	$

a. [x] A check in the amount of $ 800.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [x] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 03-3125. A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

John P. White
Cooper & Dunham LLP (Customer No. 23432)
1185 Avenue of the Americas
New York, New York 10036
United States of America

SIGNATURE

Alan J. Morrison
NAME

37,399
REGISTRATION NUMBER

65532-A-PCT-US/JPW/JW

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

the specification of which:
(check one)

X *is attached hereto.* (§371 national stage of PCT/US2003/012679, filed April 23, 2003)

___ *was filed* _____ *as*

Application Serial No. _____

and was amended on October 19, 2005
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
PCT/US2003/012679	PCT	April 23, 2003	X	___
			___	___
			___	___
			___	___
			___	___
			___	___

Revised 09/02/04

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	*Filing Date*	*Status*
60/374,929	April 23, 2002	Pending as of April 23, 2003

I hereby claim the benefits under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	*Filing Date*	*Status*
PCT/US2003/012679	April 23, 2003	

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); William E. Pelton (Reg. No. 25,702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Paul Teng (Reg. No. 40,837); Alan J. Morrison (Reg. No. 37,399); Gary J. Gershik (Reg. No. 39,992);

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White, Esq. *Reg.No.* 28,678
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Lawrence G. Lum and Randall J. Lee
U.S. Serial No.	:	Not Yet Known (national stage of PCT International Application No. PCT/US2003/012679)
Filed	:	Herewith
For	:	COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

1185 Avenue of the Americas
New York, New York 10036
October 19, 2005

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

This Preliminary Amendment is being submitted in connection with the above-identified §371 national stage application based on PCT International Application No. PCT/ US2003/012679, filed April 23, 2003, which claims priority of U.S. Provisional Application No. 60/374,929, filed April 23, 2002. Although thirty months from the priority date was October 23, 2004, the above-identified §371 national stage application is being submitted together with a Petition For Revival Of An International Application For Patent Designating The U.S. Abandoned Unintentionally Under 37 C.F.R. 1.137(b). Accordingly, this Preliminary Amendment is being timely filed.

Amendments to the Specification begin on page 2 of this paper.

A new **Abstract of the Disclosure** is referred to on page 3 of this paper and attached hereto as **Exhibit A**.

Amendments to the Claims are reflected in the listing of claims which begins on page 4 of this paper.

Remarks begin on page 8 of this paper.

Amendments to the Specification

Please replace the paragraph on page 1, lines 5-7, with the following paragraph:

> This application is a §371 national stage of PCT International Application No. PCT/US2003/012679, filed April 23, 2003, claiming priority of U.S. Provisional Application No. 60/374,929, filed April 23, 2002, the contents of all of which are hereby incorporated by reference.

Abstract of the Disclosure

Please insert new page 54 attached as **Exhibit A** hereto which sets forth an Abstract of the Disclosure.

Amendments to the Claims:

Please cancel claims 13-16, 18-23, 26-45, 47-61, 63, 64, 66-88, 90, and 91 without disclaimer or prejudice to applicants' right to pursue the subject matters of these claims in the future.

Pursuant to 37 C.F.R. §1.121(c), this listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) A composition of matter for delivering and/or affixing a stem cell to a target tissue comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

2. (Original) The composition of claim 1, wherein the first and second moieties are antigen-binding portions of an antibody.

3. (Original) The composition of claim 2, wherein the antigen-binding portions are Fab fragments.

4. (Original) The composition of claim 1, wherein the composition comprises a bi-specific antibody.

5. (Original) The composition of claim 1, wherein the composition comprises a single polypeptide chain comprising the first and the second moieties.

6. (Original) The composition of claim 1, wherein the composition comprises a recombinantly produced polypeptide chain.

7. (Original) The composition of claim 1, wherein the first

and second moieties are affixed via a chemical moiety.

8. (Original) The composition of claim 1, wherein the first and second moieties are affixed via a polypeptide moiety.

9. (Original) The composition of claim 1, wherein the stem cell is mammalian.

10. (Original) The composition of claim 9, wherein the stem cell is a $CD34^+$ cell.

11. (Original) The composition of claim 9, wherein the stem cell is an embryonic stem cell.

12. (Original) The composition of claim 1, wherein the target tissue is selected from the group consisting of hepatic tissue, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue, skin, endothelial tissue and cardiac tissue.

13-16. (Canceled)

17. (Original) A nucleic acid encoding a polypeptide for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

18-23. (Canceled)

24. (Original) A method for producing a polypeptide useful for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue, which method comprises (a) culturing the host-vector system of claim 23 under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

25. (Original) An article of manufacture for delivering and/or affixing a stem cell to a target tissue via juxtaposition of the article to the target tissue, comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface.

26-45. (Canceled)

46. (Original) A method for delivering and/or affixing a stem cell to a subject's target tissue comprising contacting the tissue with the stem cell and a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

47-61. (Canceled)

62. (Original) A method for delivering and/or affixing a stem cell to a subject's target tissue comprising, in no particular order, the steps of (a) juxtaposing to the tissue an article of manufacture comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) contacting the article with the stem cell.

63. (Canceled)

64. (Canceled)

65. (Original) A method for delivering and/or affixing a stem cell to a subject's target tissue comprising juxtaposing to the tissue an article of manufacture comprising (a) a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) the stem cell bound to the article via the composition of

matter affixed thereto.

66-88. (Canceled)

89. (Original) A composition of matter comprising (a) a stem cell to be delivered to and/or affixed to a target tissue, and (b) a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

90. (Canceled)

91. (Canceled)

Remarks

By this Preliminary Amendment, applicants have amended the specification to recite the continuing data for the above-identified application and have inserted an Abstract of the Disclosure attached hereto as **Exhibit A**. Applicants maintain that the amendments to the specification raise no issue of new matter and respectfully request that this Preliminary Amendment be entered.

Claims 1-91 are present in the application as filed. By this Preliminary Amendment, applicants have canceled claims 13-16, 18-23, 26-45, 47-61, 63, 64, 66-88, 90, and 91 without disclaimer or prejudice. Accordingly, upon entry of this Preliminary Amendment, claims 1-12, 17, 24, 25, 46, 62, 65, and 89 will be pending.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the enclosed filing fee of $800.00, is deemed necessary in connection with the filing of this Preliminary Amendment. If any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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Exhibit A

COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

ABSTRACT OF THE DISCLOSURE

This invention provides compositions of matter, articles of manufacture and methods for delivering and/or affixing a stem cell to a target tissue. This invention also provides related nucleic acids, vectors, cell, methods of production, and kits.

COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

This application claims priority of U.S. Serial No. 60/374,929, filed April 23, 2002, the contents of which are incorporated herein by reference.

Throughout this application, various references are cited. Disclosure of these references in their entirety is hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

Background of the Invention

The era of plasticity began with the publication of a manuscript entitled "Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*" by Bjornson and colleagues (1). They pursued a hypothesis based on observations made by Valtz et al. who showed the ability of a single neuroectodermal cell (from rat cerebellar cell line ST15A) to form neuronal, glial, and muscle cells (2). A deluge of plasticity papers has since followed.

Initially, there were reports of cells from muscle giving rise to hematopoiesis (3) and then a variety of reports of marrow-derived cells giving rise to muscle (4-6), hepatocytes (7-9) and cardiac myocytes (10, 11). This suggested that hierarchical plasticity is the rule and that the local microenvironment determines the choice of differentiation pathways.

While most of these studies have been done with whole cell populations, several experimental designs have used highly purified hematopoietic marrow stem cells, showing that hepatocytes and myocardial myocytes could arise from these cells. However, even in this instance, the results did not address the question of whether the repopulating cells were cells with both hematopoietic and nonhematopoietic potential or whether there coexisted separate lineage-defined stem cells in the purified population experimentally obtained. Work from Verfaille and colleagues provides support for the concept of multiple stem cell types residing in the marrow. In their *in vitro* studies, they found a class of stem cells which can give rise to neural, mesenchymal, muscle and fat cells, but not to hematopoietic lineages. The question of origin can only be answered with clonal population studies. One such study, using limiting dilution techniques, has been reported and indicates clonal origin of many nonhematopoietic cell types from purified marrow hematopoietic stem cells (12).

Another unresolved issue is whether the hematopoietic potential demonstrated in nonhematopoietic tissue arose from nonhematopoietic tissue stem cells or hematopoietic stem cells, which coexisted in the nonhematopoietic tissue. The initial reports of muscle cells generating hematopoiesis implied that muscle stem cells were responsible. However, recent work from Kawada and Ogawa (13) indicates that these initial reports simply demonstrate the existence of hematopoietic stem cells, which are known to circulate in the blood, within the muscle tissue. That study demonstrates that following

reconstitution of irradiated mice with genetically marked bone marrow cells, the cells from the muscle tissue that had reconstituted hematopoietic progeny were all of donor origin.

In vivo observations of stem cell plasticity have been extended to human cells. Almeida-Porada and coworkers (14), using a permissive, pre-immune fetal sheep engraftment model, have shown that non-purified fetal human brain cells ("neurosphere" cells) can give rise to hematopoietic, hepatic, renal and gut cells. These data clearly indicate that different tissues harbor cells with lineage potential for many other tissues, and that marrow is a particularly abundant source for these cells. They further indicate the overriding importance of specific microenvironments and their associated differentiation cues. Unfortunately, they do not as yet establish whether true hierarchical plasticity exists or whether multiple stem cells coexist in various tissues.

This model, of course, may hold for many other tissues, regardless of whether there are single or multiple stem cell types. When stem cells emigrate or are injected into a tissue, differentiation would be determined by the local environment. Thus, cardiac tissue might harbor, at least transiently, all stem cell types, but only cardiac-type stem cells would differentiate and make heart cells. Alternatively, one stem cell with open potential may be involved and its differentiation fate would then be determined by inductive signals delivered by the local environment.

There is another intriguing possibility, which is that

the marrow could actually be the feeder tissue for all other local stem cell populations. In this scenario, marrow stem cells with general potential are continuously circulating and these circulating cells would be the source of local stem cells in the gut, skin, liver or brain. Thus, the marrow would be the ultimate source of all stem cells and would feed the tissue stem cells. This would still be consistent with there being one marrow stem cell with total plastic potential or many individual stem cells with specific lineage potentials.

Notwithstanding the mechanisms of stem cell biology being studied, there exists a need for technology permitting the delivery and affixing of stem cells to particular target tissues. At present, however, such technology has not been adequately developed.

Summary of the Invention

This invention provides a first composition of matter for delivering and/or affixing a stem cell to a target tissue comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

This invention also provides a nucleic acid encoding a polypeptide for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably linked to a second moiety that specifically binds to the surface of a cell in the tissue.

This invention further provides an expression vector comprising the instant nucleic acid, and a host-vector system comprising a host cell transfected with the instant expression vector.

This invention further provides a method for producing a polypeptide useful for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably linked to a second moiety that specifically binds to the surface of a cell in the tissue, which method comprises (a) culturing the instant host-vector system under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

This invention further provides an article of manufacture

for delivering and/or affixing a stem cell to a target tissue via juxtaposition of the article to the target tissue, comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface.

This invention further provides three methods for delivering and/or affixing a stem cell to a subject's target tissue. The first method comprises contacting the tissue with the stem cell and a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably linked to a second moiety that specifically binds to the surface of a cell in the tissue.

The second method comprises, in no particular order, the steps of (a) juxtaposing to the tissue an article of manufacture comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) contacting the article with the stem cell.

The third method comprises juxtaposing to the tissue an article of manufacture comprising (a) a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) the stem cell bound to the article via the composition of matter affixed thereto.

This invention further provides a second composition of matter comprising (a) a stem cell to be delivered to and/or affixed to a target tissue, and (b) a composition of matter comprising a first moiety that specifically

binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

Finally, this invention provides two kits. The first kit comprises the first composition of matter and instructions for using same to deliver and/or affix a stem cell to a target tissue. The second kit comprises the instant article of manufacture and instructions for using same to deliver and/or affix a stem cell to a target tissue.

Detailed Description of the Invention

Definitions

As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below.

"Administering" shall mean delivering in a manner which is effected or performed using any of the various methods and delivery systems known to those skilled in the art. Administering can be performed, for example, topically, intravenously, pericardially, orally, via implant, transmucosally, transdermally, intramuscularly, subcutaneously, intraperitoneally, intrathecally, intralymphatically, intralesionally, or epidurally. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

The term "antibody" includes, by way of example, both naturally occurring antibodies (e.g., IgG, IgM, IgE and IgA) and non-naturally occurring antibodies. The term "antibody" also includes polyclonal and monoclonal antibodies, and fragments thereof (e.g., antigen-binding portions). Furthermore, the term "antibody" includes chimeric antibodies, wholly synthetic antibodies, human and humanized antibodies, and fragments thereof.

"Host cells" include, but are not limited to, bacterial cells, yeast cells, fungal cells, insect cells, and mammalian cells. Mammalian cells can be transfected by methods well-known in the art such as calcium phosphate

precipitation, electroporation and microinjection.

"Mammalian cell" shall mean any mammalian cell. Mammalian cells include, without limitation, cells which are normal, abnormal and transformed, and are exemplified by neurons, epithelial cells, muscle cells, blood cells, immune cells, stem cells, osteocytes, endothelial cells and blast cells.

The terms "nucleic acid", "polynucleotide" and "nucleic acid sequence" are used interchangeably herein, and each refers to a polymer of deoxyribonucleotides and/or ribonucleotides. The deoxyribonucleotides and ribonucleotides can be naturally occurring or synthetic analogues thereof.

"Pharmaceutically acceptable carriers" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers can be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions and suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present,

such as, for example, antimicrobials, antioxidants, chelating agents, inert gases, and the like.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein, and each means a polymer of amino acid residues. The amino acid residues can be naturally occurring or chemical analogues thereof. Polypeptides, peptides and proteins can also include modifications such as glycosylation, lipid attachment, sulfation, hydroxylation, and ADP-ribosylation.

"Specifically bind" shall mean that, with respect to the binding of a moiety to the surface of a cell, the moiety binds to that cell with a greater affinity than that with which it binds to the surface of most or all other cells. In the preferred embodiment, the moiety binds to that cell with a greater affinity than that with which it binds to the surface of all other cells.

"Stem cell" shall mean, without limitation, a cell that gives rise to a lineage of progeny cells. Examples of stem cells include CD34+ cells, CD45+ cells and embryonic stem cells. Surface adhesion molecules present on stem cells include, without limitation, IL-3 receptor, IL-6 receptor, IL-11 receptor, c-kit, VLA-4, VLA-5, L-selectin, PECAM-1 and Beta-1 integrin.

"Subject" shall mean any animal, such as a mammal or a bird, including, without limitation, a cow, a horse, a sheep, a pig, a dog, a cat, a rodent such as a mouse or rat, a turkey, a chicken and a primate. In the preferred embodiment, the subject is a human being.

"Target tissue" shall mean any biological tissue to which stem cell delivery and/or attachment is desired. Target tissue includes, without limitation, normal, damaged and diseased tissue.

"Vector" shall mean any nucleic acid vector known in the art. Such vectors include, but are not limited to, plasmid vectors, cosmid vectors, and bacteriophage vectors.

Embodiments of the Invention

This invention provides two compositions of matter. The first composition of matter is for delivering and/or affixing a stem cell to a target tissue which comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

The second composition of matter is for delivering and/or affixing a stem cell to a target tissue which comprises (a) the stem cell and (b) a composition of matter which comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

The first and second moieties can be of any type. In one embodiment of the instant compositions, the first and second moieties are antigen-binding portions of an antibody. Antigen-binding portions include, for example, Fab fragments.

In the instant compositions, the compositions can also comprise bi-specific antibodies. Moreover, these compositions can comprise a single polypeptide chain comprising the first and the second moieties. Further, the instant compositions can comprise a recombinantly produced polypeptide chain.

In the instant compositions, the first and second moieties can be affixed via any suitable means. In one embodiment, the first and second moieties are affixed via a chemical moiety. In another embodiment, the first and second moieties are affixed via a polypeptide moiety.

In the instant compositions, the stem cell can be any stem cell. In one embodiment, the stem cell is mammalian. Mammalian stem cells include, for example, stem cells from a cow, a horse, a sheep, a pig, a dog, a cat, a rodent and a primate. Preferably, the stem cell is human. Stem cells used in the instant invention also include, by way of example, $CD34^+$ cells and embryonic stem cells.

In the instant compositions, the target tissue can be any suitable target tissue including, for example, hepatic tissue, skin, epithelial tissue, connective tissue, articular tissue, bone tissue (including, for example, bone marrow and other hematopoietic cells), muscle tissue, neuronal tissue and cardiac tissue. In one embodiment, the target tissue is cardiac tissue. In another embodiment, the target tissue is skin. Cardiac tissue can be abnormal, and includes, without limitation, diseased myocardial tissue, damaged myocardial tissue,

diseased valve tissue, damaged valve tissue, diseased cardiovascular tissue and damaged cardiovascular tissue. Likewise, skin can be abnormal and includes, without limitation, diseased and damaged skin. Moreover, biochemical features of the target tissue recognized by the target tissue-binding moieties of the instant compositions include, by way of example, myosin, cardiac troponin T, cardiac troponin I, actin, beta-myosin heavy chain, tropomyosin or any other feature to which such moieties can be directed.

In another embodiment of the instant compositions, the compositions further comprise a pharmaceutically acceptable carrier.

This invention also provides a nucleic acid which encodes a polypeptide that binds to a stem cell. In one embodiment, the nucleic acid is DNA or RNA, and in another embodiment, the nucleic acid is DNA.

The instant nucleic acid can be an expression vector. In one embodiment, the vector is selected from a plasmid, a cosmid, a bacteriophage and a eukaryotic virus. Eukaryotic viruses include, for example, adenoviruses and retrovirus.

This invention further provides a host-vector system comprising a host cell transfected with the instant expression vector.

This invention further provides a method for producing a polypeptide useful for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a

first moiety that specifically binds to the stem cell surface operably linked to a second moiety that specifically binds to the surface of a cell in the tissue, which method comprises (a) culturing the instant host-vector system under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

This invention further provides a first article of manufacture for delivering and/or affixing a stem cell to a target tissue via juxtaposition of the article to the target tissue, comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface.

In a preferred embodiment of this invention, the solid substrate is biodegradable. In another embodiment, the solid substrate comprises a polymer, such as teflon. In a further embodiment, the solid substrate comprises an agent selected from fibrin, vicryl, hyaluronic acid, polyethylene glycol, polylactic acid, polylactic-co-glycolic acid, collagen, thrombospondin, teflon, osteopontin and fibronectin.

The instant article can be in any suitable physical form including, for example, gauze, a bandage, suture, a stent, an implant or a polymeric matrix.

Preferably, the composition of matter affixed to the solid substrate further comprises a second moiety that specifically binds to the surface of a cell in the tissue. In one embodiment, the moiety is an antigen-

binding portion of an antibody, such as a Fab fragment. In another embodiment, the composition comprises a bi-specific antibody. In a further embodiment, the composition comprises a single polypeptide chain comprising the first and the second moieties. In still a further embodiment, the composition comprises a recombinantly produced polypeptide chain.

In the instant article, the first and second moieties can be affixed by any suitable means, such as via a chemical moiety and via a polypeptide moiety.

In the instant article, the stem cell can be any stem cell. In one embodiment, the stem cell is mammalian. Mammalian stem cells include, for example, stem cells from a cow, a horse, a sheep, a pig, a dog, a cat, a rodent and a primate. In another embodiment, the stem cell is avian. Avian stem cells include, for example, turkey and chicken stem cells. Preferably, the stem cell is human.

Also in the instant article, the target tissue can be any suitable target tissue including, for example, hepatic tissue, skin, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue and cardiac tissue. In one embodiment, the target tissue is cardiac tissue.

This invention further provides a second article of manufacture intended for the affixation of stem cells to the article's surface, comprising a solid substrate having on its surface a moiety which is specifically bound by a composition of matter which also specifically

binds to a stem cell.

The various embodiments of the first article of manufacture, such as the nature and physical form of substrate, are envisioned, as applicable, for the second article of manufacture.

This invention further provides three methods for delivering and/or affixing a stem cell to a subject's target tissue. The first method comprises contacting the tissue with the stem cell and a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

In one embodiment of the first method, the contacting is performed *ex vivo*. In another embodiment, the contacting is performed *in vivo*.

In another embodiment of the first method, the stem cell and composition of matter are first contacted with each other so as to permit the formation of a complex therebetween, and the resulting complex is contacted with the target tissue. The complex can be contacted with the target tissue via any suitable means, such as topical or intravenous administration.

In the first method, the first and second moieties can be of any type. In one embodiment, the first and second moieties are antigen-binding portions of an antibody. Antigen-binding portions include, for example, Fab fragments.

Also in the first method, the composition can comprise a bi-specific antibody. Moreover, this composition can comprise a single polypeptide chain comprising the first and the second moieties. Further, the composition can comprise a recombinantly produced polypeptide chain.

In the first method, the first and second moieties can be affixed via any suitable means. In one embodiment, the first and second moieties are affixed via a chemical moiety. In another embodiment, the first and second moieties are affixed via a polypeptide moiety.

The second method for delivering and/or affixing a stem cell to a subject's target tissue comprises, in no particular order, the steps of (a) juxtaposing to the tissue an article of manufacture comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) contacting the article with the stem cell.

In one embodiment of the first method, the article is contacted with the stem cell via intravenous administration of the stem cell. In one embodiment of the second method, the article is contacted with the stem cell via topical administration of the stem cell.

The third method for delivering and/or affixing a stem cell to a subject's target tissue comprises juxtaposing to the tissue an article of manufacture comprising (a) a solid substrate having operably affixed thereto a composition of matter comprising a moiety that

specifically binds to the stem cell surface, and (b) the stem cell bound to the article via the composition of matter affixed thereto.

In the instant methods, the article can be in any suitable physical form including, for example, gauze, a bandage, suture, a stent or a polymeric matrix.

Preferably in the instant methods, the composition of matter affixed to the solid substrate further comprises a second moiety that specifically binds to the surface of a cell in the tissue. In one embodiment, the moiety is an antigen-binding portion of an antibody, such as a Fab fragment. In another embodiment, the composition comprises a bi-specific antibody. In a further embodiment, the composition comprises a single polypeptide chain comprising a first and a second moiety that specifically bind to the stem cell surface and tissue cell surface, respectively. In still a further embodiment, the composition comprises a recombinantly produced polypeptide chain.

In the instant methods, the first and second moieties can be affixed by any suitable means, such as via a chemical moiety and via a polypeptide moiety.

The subject in the instant methods can be any subject. Likewise, the stem cell can be a stem cell from any subject. In one embodiment, the subject is a mammal. Mammals include, for example, a cow, a horse, a sheep, a pig, a dog, a cat, a rodent and a primate. In another embodiment, the subject is a bird. Birds include, for example, turkeys and chickens. Preferably, the subject

is human, and the stem cell is human.

In the instant methods, the target tissue can be any suitable target tissue including, for example, hepatic tissue, skin, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue and cardiac tissue. In one embodiment, the target tissue is cardiac tissue.

Finally, this invention further provides kits for delivering and/or affixing a stem cell to a target tissue. The first kit comprises the first instant composition of matter and instructions for use. The second kit comprises the instant article of manufacture and instructions for use.

This invention will be better understood from the Examples that follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Examples

Example 1

Examples of Therapeutic Indications for Stem Cell Therapy

Organ failure states

Cardiac disorders (e.g., myocardial infarction, valvular disease, hypertrophic/restrictive diseases, myocarditis and cardiomyopathy); kidney failure, acute and chronic; liver failure, acute and chronic; lung failure, acute and chronic; COPD and ARDS; and skin failure such as dermatological disorders, diabetic ulcers, burns, chemotherapy or radiation damage, and cancer-related skin damage.

Neurologic disorders

Alzheimer's and other degenerative disorders; structural damage to brain or spinal cord from stroke or trauma; peripheral neuropathy; and degenerative disorders (e.g., Amyotrophic Lateral Sclerosis (Lou Gehrig's disease) and Guillain-Barre syndrome).

Malignancies

Primary bone marrow disorders (e.g., leukemia, myelodysplastic syndrome); and solid tumors.

Autoimmune diseases

Systemic lupus erythematosus, eczema, psoriasis, and ITP.

Genetic-based diseases

Sickle cell anemia, thalassemia, hemoglobinopathies, and hemophilia.

All known stem cell diseases

Chronic myelogenous leukemia; most acute leukemias; polycythemia rubra vera, primary thrombocytosis; myelofibrosis with myeloid metaplasia, aplastic anemia; paroxysmal nocturnal hemglobinuria; most lymphomas and multiple myeloma; many chronic neutropenias; pure red cell aplasia and Fanconi's anemia; and cyclic neutropenias and Shwachman-Diamond syndrome.

Example 2

The Potential of Skin Stem Cells Arising from Bone Marrow

Introduction

A number of tissue stem cell systems have been described. The hematopoietic system has perhaps been most extensively characterized. The hematopoietic stem cell has been felt to be a cell with extensive proliferative, renewal and differentiative potential for red cell, white cell and platelet lineages. In similar fashion, stem cells in intestinal crypts, hippocampal, subventricular zone, neural crest, and eye conjunctival have also been shown to produce major cell types in their respective organs. Less well-characterized stem cell systems have been reported for muscle and liver. Hair follicle bulge stem cells produce epithelial cells, cells for the outer root sheath and matrix as well as lipid-producing

sebaceous glands. Epidermal skin stem cells have been partially characterized by cell surface markers, size and *in vitro* adhesion characteristics. Both label-retaining and transient amplifying populations have been described. Isolation of skin stem cells using cell size and Hoechst red/blue dye exclusion has recently been described. This technique is an adaptation of a well-characterized method for isolating hematopoietic stem cells. In a recent study, stem cells in skin dermis have also been described.

Recently, it has been appreciated that stem cells may show tremendous plasticity and that a stem cell from one tissue may commit to a different fate when located in a different tissue. There has followed a large number of reports showing that muscle and hepatic cells can make blood cells, that adipose cells can differentiate into chondrocytic, myogenic and osteogenic lineages and that marrow cells can produce a wide variety of cell types. Marrow has now been shown to be capable of producing, *in vitro* and *in vivo*, hepatic, renal, pulmonary, gastrointestinal, neural, chondrocyte, adipocyte, cardiac and skeletal muscle, as well as bone cells. Two particularly impressive studies have shown highly purified murine stem cells to be capable of producing hepatic cells or cardiac myocytes and of reversing disease manifestations in these organs. Recently, Ogawa and colleagues have published data indicating that the skeletal muscle stem cells, which were reported as having hematopoietic potential, may have originally derived from marrow.

These studies raise important questions as to the source

of many, or possibly all tissue stem cells. One possibility is that the marrow could be such a source. Marrow stem cells are continuously present in the peripheral blood and it is now known that marrow cells appear to have the capacity for generating many other cell types when residing in a specific tissue. Marrow cells may continuously renew tissue stem cells through the lifespan of an animal. Renewal of resident stem cells may be required for maintenance of an organ and for repair of damage due to injury. This may be of particular importance in highly regenerative organs such as the liver or skin, which are tissues very familiar with injury due to toxic insult or wounding. It is believed that wounding is in fact a mechanism by which stem cells are recruited to skin.

The failure of chronic wounds to heal may be due in part to the loss or malfunction of resident skin stem cells. This notion does not require a great leap of faith, as recent studies (data not shown) have shown that cells derived from chronic wounds appear altered in their growth capacity and in their ability to respond to certain cytokines. Cultured dermal fibroblasts appear senescent, as shown by their decreased capacity to undergo population doubling and by other parameters. Lower extremities from patients with advanced arterial and/or venous disease are also noted to have decreased numbers of hair follicles, which are the predominant source of epidermal skin stem cells in that body location. This reduction of follicles would then represent a loss of resident stem cells in the vicinity of chronic wounds.

The ability to bring new young cells into the wound is generally thought to explain the effectiveness of bioengineered skin in treating chronic wounds. Recent work (data not shown) has shown that delivery of bone marrow stem cells to wounds reverses the failure of chronic wounds to heal and promotes rebuilding of the dermal structures.

Many of these new observations have come about because of the availability of specific markers to track cell populations and labeling techniques to identify the nature of donor cells in a transplanted mouse. For example, markers for repetitive sequences on the Y chromosome recently became available allowing for the tracking of male cells in female hosts, especially in strains which do not show HY immunoreactivity. Both Southern blot and fluorescent-in-situ-hybridization (FISH) were utilized in those experiments. The availability of transgenic mice with specific markers has allowed for rapid progress in this field. The most commonly used systems have been markers for green fluorescent protein or for expression of β-galactosidase. Rosa mice transgenic for ß-galactosidase expression have been used in many studies, while a number of GFP-transgenics have also been used.

Results

In the past, it was shown that marrow cells were able to give rise to bone cells when infused at relatively high levels (over 80 million) into non-ablated host mice. Morphology and FISH on serial marrow sections were used. Here, the marrow sections were prepared in a unique

fashion using anesthetized mice and low-pressure paraformaldehyde infusion through the descending aorta.

More recently, a model was evaluated for transplanting green-fluorescent protein (GFP)-positive transgenic marrow cells into hosts and evaluating immediate homing and eventual cell fate in the skin and other tissues. In these studies, GFP+ transgenic mice were used as donors and C57BL/6J mice (same sex) were used as hosts. Host mice were exposed to 400 cGy whole body irradiation and then infused with 25 million GFP+ marrow cells. In some experiments CFDA-SE, a cytoplasmic nonspecific fluorescent probe, was also used to label the marrow cells. Cohorts of mice were maintained for 3 months and peripheral blood chimerism was assessed at different intervals. A stable chimerism of between 70-80% was achieved.

Three months post-marrow cell infusion mice were divided into 3 groups. One group was not further treated. The other two groups received two excisional wounds (per mouse) on the back. These wounded groups differed in that one group was given G-CSF twice daily for 4 consecutive days before wounding and on the day of wounding (total 5 days). The time of excisional wounding was counted as day 0 for all groups. On day 2, the non-wounded group had a skin biopsy performed on the back and the two wounded groups had one of their excisional wounds harvested for analysis. On day 21, the non-wounded group had a skin biopsy performed on the back and the two wounded groups had their remaining excisional wounds harvested for analysis. The tissues were evaluated for the presence of donor cells and for the phenotype of the

donor cells.

There were several GFP+ cells in the dermis of the transplanted unwounded mice at both time points. This finding supports the notion that there is constant trafficking of bone marrow cells to the dermis. The spindle cell and round morphologies of these cells could indicate that these cells may be inflammatory in nature. However, the H&E stained companion sections did not reveal a significant inflammatory infiltrate. Rather, these cells appear to have a fibroblast or tissue macrophage like morphology. The number of GFP+ dermal cells in non-wounded transplanted mice was slightly higher than that in sections prepared from the skin of donor GFP+ mice. This finding might be a secondary effect of the radiation to which the transplanted mice were exposed. The effect of radiation could have been to locally reduce the number of resident progenitor cells. This may have created "room" for the bone marrow cells to repopulate the area.

In the wounded mice at day 2, there was a significant inflammatory infiltrate in both groups. In the G-CSF-treated group, the inflammatory infiltrate was much greater than in the non-G-CSF-treated group. The amount of GFP present in the wound due to the infiltrate and ruptured inflammatory cells obliterated the wound field with fluorescence in many cases. Interpretation of these sections for engraftment of cells was difficult in both wounded groups due to the high level of signal present.

At day 21, the amount of inflammation in the wounded groups was mostly resolved. There did not seem to be a significant difference in the number of GFP+ cells in both wounded groups. Several GFP+ mature (and immature) blood vessels were noted in the dermis of both wounded groups. There were GFP+ cells noted in the striated muscle of the dermis, hair follicle, sebaceous glands and epidermis in both wounded groups. However, there seemed to be more GFP+ cells in the epidermis, hair follicles and sebaceous glands of the G-CSF-treated mice. Hair follicle, sebaceous gland and epidermal GFP+ cells were also shown to double label for keratin and GFP antibodies. These findings strongly support the idea that bone marrow may supply needed stem and/or progenitor cells to wounded cutaneous tissues.

Recently, chronic ulcers of greater than one-year duration were treated with autologous bone marrow derived cells. The patients selected had failed a number of sophisticated wound care treatments in an advanced wound care clinic. These prior treatments included autologous skin grafting and grafting with bioengineered skin. Biopsies obtained from these patients indicate that bone marrow cells engrafted into the wounds. To date, all patients treated with bone marrow cells are currently healed. As described above, it is well known that chronic wounds have an altered local environment with evidence of cell senescence and depletion of resident stem and/or progenitor cells. The instant work illustrates the significance of bone marrow in delivering stem and/or progenitor cells to wounds.

Example 3

Cell-Cycle Related Stem Cell Homing and Transdifferentiation

Introduction

Recent studies have indicated that marrow-derived stem cells have the capacity to home to and differentiate in nonhematopoietic tissues producing cells with nonhematopoietic lineages typical of that tissue, i.e., so-called transdifferentiation. In several studies, highly purified murine marrow stem cells were shown to repopulate diseased or injured hepatic and cardiac tissue, respectively, and were also shown to restore or improve function in these tissues. Thus, marrow stem cells evidence a remarkable plasticity with regard to making nonhematopoietic cells.

Others have studied a different type of marrow stem cell plasticity; that of cell cycle-related shifts in engraftment or differentiation phenotype of the stem cell. These studies suggested that when purified marrow stems are induced to transit cell cycle, they reversibly alter their adhesion protein profile that in turn effects homing. This homing then determines the results of engraftment in marrow.

Data

The above-described sequence of events was demonstrated when marrow was cultured with interleukin (IL)-3, IL-6, IL-11 and steel factor. Marrow cells were studied in standard static tissue culture conditions and in simulated microgravity using NASA-supplied rotating

tissue culture vessels. These studies investigated both the impact of cycle progression induced by the cytokine cocktail IL-3, IL-6, IL-11 and steel factor or alternatively by thrombopoietin (TPO), FLT-3 ligand (FLT-3L) and steel factor on marrow cell engraftment and differentiation.

The results of these studies were as follows. (1) Growth under microgravity conditions appears to favor support of relatively differentiated cells. (2) Shifts of engraftment phenotype were seen with cytokine induced cell cycle transit under normal and microgravity conditions. (3) These engraftment phenotype shifts were reversible and in each case appeared tied to cell cycle. (4) Shifts in the differentiation phenotype were seen at points in cell cycle which differed from the shifts in the engraftment phenotype and which were also reversible. These latter observations are particularly important, in that they suggest the presence of differentiation "hotspots" at different points in the cell cycle. At certain points in the cell cycle, the purified cells ($Lineage^{negative\ (-)}$ Rhodamine (Rho) ^{low}Hoechst (Ho) 33342^{low}) present the phenotype of a primitive engraftable stem cell while at other times the phenotype is that of a progenitor. This further suggests that there is no stem cell/progenitor hierarchy but rather a fluctuating continuum with continual and reversible changes in phenotype tied to the phase of the cell cycle.

Example 4

Bispecific Antibody Targeting of Stem Cells to Nonhematopoietic Tissues

Background and Results

Bispecific antibodies can be constructed by genetic engineering or chemical conjugation techniques. In recent studies, bispecific antibodies that link CD3 and HER2/*neu* were chemically conjugated which selectively bind T cells to HER2/*neu*-expressing tumor cells. It was shown that this binding results in significantly increased cytotoxic functions of T cells to breast cancer cells. Further work on molecular engineering of bispecific antibodies has shown that T cells armed with the recombinant protein containing only the scFv portions of two antibodies can specifically target and kill the tumor cells.

Recent studies indicate that marrow stem cells can give rise to a variety of cell types in different tissues and rapidly correct tissue dysfunction *in vivo*, and suggest that targeting of marrow stem cells to particular tissues could increase the efficiency of marrow-derived transdifferentiation in these tissues.

Project 1

The purpose of this project is to produce a bispecific monoclonal antibody (BsAb), anti-VCAM-1 x anti-c-Kit (named VK-Bi), that will target marrow stem cells to skin.

Bi-specific antibodies are created which link primitive murine lymphohematopoietic stem cells ($Lin^-Rho^{low}Ho^{low}$, Lin-Sca-1+, or Lin-Hoechst side population) to skin cells expressing the following injury ligands; VCAM-1, ICAM-1, P-selectin, IL-8 or P63. C-kit has been selected for the stem cell epitope because in recent studies, it was shown to be universally present on $Lin^-Rho^{low}Ho^{low}$ and Ho^{low} and Lin-Sca+ murine marrow hematopoietic stem cells and because it has been used by a number of investigators to isolate homing and engrafting stem cells, i.e., binding of antibody to c-kit does not appear to interfere with stem cell homing and engraftment. Thus, the first bispecific antibody that is prepared, characterized and validated as a reagent is a heteroconjugate that binds on one end to c-kit and the other end to VCAM-1.

These two antibodies are conjugated through two reagents, the Traut and SMCC. The procedure of BsAb production has been well established. This heteroconjugation reaction includes two steps. (1) Anti-c-Kit is cross-linked with 10-fold molar excess of Traut reagent (2-iminothiolane HCL, Pierce) and (2) anti-VCAM-1 with 4-fold molar excess of SMCC (sulphosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate, Pierce). The Traut Buffer contains 50 mM NaCl, 1 mM EDTA, pH 8.0 and the SMCC buffer, 0.1 M sodium phosphate, 0.15 M NaCl at pH 7.2. The cross-linking reaction takes place at room temperature for one hour. The cross-linked antibody is then purified on a PD-10 column (Pharmacia, Uppsala, Sweden) in PBS to remove unbound cross-linker. In the second step, the cross-linked antibodies are mixed immediately at an equal molar ratio and conjugated at 4°C overnight on a shaker. Control bispecific antibodies are

also created to evaluate the specificity of binding. Here, anti-c-kit and anti-irrelevant antibodies are used, e.g., anti-CD2.

Heteroconjugation products are confirmed by non-reducing SDS PAGE gels. The specificities of the bispecific antibody product is verified by studies of the ability of the bispecific antibody to bind stem cells to VCAM-1-expressing cells.

The unfractionated preparations of anti-c-Kit x anti-VCAM-1 contain monomers, dimers and multimers. Only 20-30% of heteroconjugation products are in dimer form. The total antibody mixture is assessed, including multimers, dimers and monomers. This mixture has been used effectively in the above-cited OKT3/HER2/neu bispecific antibody studies. The efficacy of dimer/multimer fractions purified by sizing chromatography is also assessed. This, of course, gives a more purified characterized reagent, but at the cost of significant loss of antibody (approximately 80-90% based on previous experience). This reagent should be sufficiently pure. The isolation of dimer free from multimer may be accomplished by use of ion exchange chromatography, but this would be at the cost of significant further loss of antibody. Perhaps the critical observation here is that the original preparation of bispecific antibody to OKT3/HER2/neu, which had multimers, dimers and monomers, effectively bound T cells to breast cancer cell line cells. Furthermore, in studies in which multimers, dimers and monomers were separated, binding activity was found in the dimer and multimer fractions.

Project 2

The purpose of this project is to evaluate the function of anti-c-Kit x anti-VCAM-1 *in vitro* using skin cell lines and biopsy skin tissues, and *in vivo* using the mouse model.

Specifically, this work is to determine whether a bispecific antibody binds to c-kit on purified Lin^- $Rho^{low}Ho^{low}$ or Lin-Sca+ or Lin-Hoechst side population marrow stem cells from male BALB/c or Rosa beta-galactosidase-positive mice, and mediates increased binding of the stem cells to epithelial cells expressing the injury ligands VCAM-1, ICAM-1, P63, E-selectin and IL-8.

Binding of the anti-c-kit x anti-VCAM-1 bispecific antibody to different skin cells and c-kit+ populations from different sources is evaluated. In *in vitro* studies, the bispecific antibodies are titrated in VCAM-1+ injured skin cells (not expressing c-kit) and to c-kit+ stem cells (not expressing VCAM-1). An irrelevant bispecific antibody is used as a control.

The *in vivo* homing of c-kit cells labeled with the non-specific fluorescent dye, CFDA-SE and with bound bispecific antibody to normal, biopsied or locally irradiated skin is monitored. These studies include homing, subsequent cell fate and determination of trans-differentiation.

Using established animal transplantation models, it is determined whether the bispecific antibody can augment the homing process of stem cells and enhance the differentiation in the target environment (the injured skin). In brief, beta-galactosidase-positive Rosa mice are used as marrow stem cell donors and C57BL/6J mice (same sex) are used as hosts. For the homing studies, the cytoplasmic dye CFDA-SE is used in tissue sections and fluorescent events enumerated.

In order to follow cell fate and possible "transdifferentiation", the intrinsic and invariant cell labels of either male DNA (the male to female BALB/c marrow transplant model) or beta-galactosidase (the Rosa to C57BL/6J marrow transplant model) are employed. This approach is necessary, because the CFDA-SE fluorescent label will be lost with continued proliferation. In these studies, double labeling studies are carried out. For male DNA, the presence of Y chromosome DNA is first determined using FISH for male sequence. These preparations are photographed and then restained for cell type-specific markers, mainly cytokeratins. These preparations are also photographed and the photos matched to determine double labeling. With the beta-galactosidase system, the sequence is reversed, first determining antibody staining and then the presence of beta-galactosidase by either x-gal staining or by anti-beta-galactosidase antibody staining.

These studies allow one to determine the capacity of murine marrow-derived stem cells with bound bispecific antibody to home to skin and then produce epithelial or other skin-associated cells. In these studies, homing and

cell fate are also determined when stem cells are untreated or bound to an irrelevant bispecific antibody. Homing and cell fate are determined in normal mice or in mice which have been subjected to a skin wound or local skin irradiation (500-2000 cGy). These injuries will occur from one day to two weeks prior to cell infusion.

Example 5
Heart Injury Model

Project 1

A myocardial ligation model was established in mice, and has use for studying tissue injury repair. C57BL/6 animals underwent coronary artery ligation followed by injection of 40 x 10^6 bone marrow cells 24, 48 and 72 hours after injury. Animals were evaluated for transdifferentiation of GFP+ cells in heart sections at different time points after injury (up to one month). There was no evidence of GFP+ myocardial cells. In a different set of experiments, C57BL/6 animals were exposed to 500cGy, followed by infusion of 25 million bone marrow cells from GFP transgenic mice. Two months later, their anterior descending coronary arteries were ligated, and after four days, the animals were injected with G-CSF to mobilize their bone marrow stem cells. The data show that in the mobilized animals, GFP+ myocardial cells can be identified (data not shown).

Project 2

Studies were conducted to see whether arming marrow cells with anti-c-kit x anti-VCAM1 bispecific antibody helps

target marrow cells to an injured heart and to determine which c-kit+ cell population would be the appropriate population for cardiac homing. These studies compared the homing performance of Lin- cells and Lin-Sca+ cells armed with control and bispecific antibody.

In studies using Lin-Sca+ purified (250,000 cells injected) cells, there was no difference between the control and the bispecific antibody at 14 hours post-infusion. However, when using Lin- cells (450,000 cells injected), there was a significant increase in homing of marrow cells armed with anti-c-kit x anti-VCAM1 to the injured heart (Data not shown).

This illustrates that a partially purified population of marrow cells at a specific time post-infusion is enhanced in its targeting to the injured heart by arming with the marrow cell with an anti-c-kit x anti-VCAM1 bispecific antibody.

Project 3

Studies were conducted to determine whether purified Lin-Sca+ cells armed with anti-c-kit x anti-VCAM1 bispecific antibody would be retained after direct intramyocardial injections or would home to injured myocardium after intravenous injection in C57BL/6 mice following myocardial infarct surgery. Two mice were anesthesized with intraperitoneal injections of ketamine and xylazine, intubated and ventilated using a Harvard rodent respirator. A midline sternotomy was performed and a 7-0 Ticron coated suture was used to tie off and occlude the apical portion of the left anterior descending artery

(LAD). The sternum and skin were closed with interrupted sutures. The mice were allowed to recover for 3 days. On the third day under isoflurane anesthesia, the mice had cut-downs performed on the right internal jugular (i.j.) vein. One mouse received 200,000 Lin-Sca+ purified cells armed with 500 ng of anti-c-kit x anti-VCAM-1/million cells and the second mouse received 200,000 unarmed Lin-Sca+ purified cells. Both the armed and unarmed Lin-Sca+ cells were labeled with CFDA-SE prior to i.j. injection.

Two other mice underwent the same infarct surgery as described above. One of the latter two mice received a direct intramyocardial injection of 200,000 CSFDA-SE labeled Lin-Sca+ cells armed with anti-c-kit x anti-VCAM1 and one received a direct intramyocardial injection of 200,000 CSFDA-SE labeled unarmed Lin-Sca+ cells. All four mice were sacrificed 6 days after their infarcts were performed and their hearts were excised, washed in saline, frozen in OCT and cryosectioned, mounted on Superfrost plus slides and viewed under fluorescence microscopy.

In the direct injection of armed Lin-Sca+ cells, the results show enhanced numbers of fluorescent cells at the site of injection in the infarct area over background auto-fluorescence at high magnification (data not shown). In the mouse that received armed CSFDA-SE labeled Lin-Sca+ cells via i.j. injection, there was clearly enhanced fluorescence with increased numbers of cells in the infarct zone that concentrated in the endocardial layers extending to epicardial layers (data not shown). In contrast, there was much less fluorescence in the infarct

area in the mouse that received CSFDA-SE labeled unarmed Lin-Sca+ cells via i.j. injection.

This shows that armed Lin-Sca+ cells injected via the internal jugular vein can home to injured myocardium whereas unarmed Lin-Sca+ do not home to injured myocardium.

Project 4

Studies were conducted using human T cells armed with OKT3 (anti-human CD3) x anti-rat ICAM1 to confirm trafficking mediated by the targeting antibody. This was a proof of principle experiment that obviated the need for purifying and sorting large numbers of Lin-Sca+ purified stem cells from the bone marrow of thirty mice. Only 1-2 million Lin-Sca+ cells can be obtained from an all-day purification process. On the other hand, large numbers of homogenous anti-CD3 activated T cells grown in low dose IL-2 can be obtained and used as living "markers" for homing to target tissue that can be easily identified by staining to T cells using CY3 fluorochrome.

The purpose of this project is two-fold: (1) to find out whether arming cells with target-specific antibodies aids in the delivery of cells to the target organ via intravenous injection, and (2) to see whether arming cells with target-specific antibodies leads to higher cell retention after direct injection into the target organ.

Four groups of nude rats which had a 17-minute infarction followed by reperfusion 1 day prior to cell injection

were used for this study. Infarction was caused by a transient ligation of their left anterior descending portion (LAD) of the left coronary artery. After 17 minutes, ligation was stopped to allow reperfusion. The animals were injected as follows: (1) i.j. injection of activated human T cells armed with mouse anti-rat ICAM1 x mouse anti-human OKT3 bispecific antibody; (2) i.j. injection of activated human T cells armed with hamster anti-mouse ICAM1 x mouse anti-human OKT3 bispecific antibody (control); (3) Direct myocardial injection of activated human T cells armed with mouse anti-rat ICAM1 x mouse anti-human OKT3 bispecific antibody; and (4) Direct myocardial injection of activated human T cells armed with hamster anti-mouse ICAM1 x mouse anti-human OKT3 bispecific antibody (control). The animals were then sacrificed 1 day later. Fresh frozen specimens of their ventricles were cryosectioned and then stained with immunofluorescent anti-mouse IgG antibodies conjugated to Cy3 to label armed cells with mouse-derived antibodies.

There was a marked difference between the two i.j. injection groups. The experimental group (activated human T cells armed with mouse anti-rat ICAM1 x mouse anti-human OKT3 bispecific antibody) showed a significant increase in immunofluorescence and cellularity relative to the control group (data not shown). These findings show that arming cells with target-specific antibodies help markedly increase the i.j. delivery of the armed cells to the target organ. However, unlike i.j. injections, arming cells with target-specific antibodies does not lead to a higher retention of armed cells after direct myocardial injections (data not shown).

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What is claimed is:

1. A composition of matter for delivering and/or affixing a stem cell to a target tissue comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

2. The composition of claim 1, wherein the first and second moieties are antigen-binding portions of an antibody.

3. The composition of claim 2, wherein the antigen-binding portions are Fab fragments.

4. The composition of claim 1, wherein the composition comprises a bi-specific antibody.

5. The composition of claim 1, wherein the composition comprises a single polypeptide chain comprising the first and the second moieties.

6. The composition of claim 1, wherein the composition comprises a recombinantly produced polypeptide chain.

7. The composition of claim 1, wherein the first and second moieties are affixed via a chemical moiety.

8. The composition of claim 1, wherein the first and second moieties are affixed via a polypeptide moiety.

9. The composition of claim 1, wherein the stem cell is mammalian.

10. The composition of claim 9, wherein the stem cell is a $CD34^+$ cell.

11. The composition of claim 9, wherein the stem cell is an embryonic stem cell.

12. The composition of claim 1, wherein the target tissue is selected from the group consisting of hepatic tissue, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue, skin, endothelial tissue and cardiac tissue.

13. The composition of claim 12, wherein the tissue is cardiac tissue.

14. The composition of claim 13, wherein the cardiac tissue is abnormal.

15. The composition of claim 14, wherein the abnormal cardiac tissue is selected from the group consisting of diseased myocardial tissue, damaged myocardial tissue, diseased valve tissue, damaged valve tissue, diseased cardiovascular tissue and damaged cardiovascular tissue.

16. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

17. A nucleic acid encoding a polypeptide for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

18. The nucleic acid of claim 17, wherein the nucleic acid is DNA or RNA.

19. The nucleic acid of claim 18, wherein the nucleic acid is DNA.

20. The nucleic acid of claim 17, wherein the nucleic acid is an expression vector.

21. The nucleic acid of claim 20, wherein the vector is selected from the group consisting of a plasmid, a cosmid, a bacteriophage and a eukaryotic virus.

22. The nucleic acid of claim 21, wherein the eukaryotic virus is an adenovirus or a retrovirus.

23. A host-vector system comprising a host cell transfected with the expression vector of claim 20.

24. A method for producing a polypeptide useful for delivering and/or affixing a stem cell to a target tissue, which polypeptide comprises a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue, which method comprises (a) culturing the

host-vector system of claim 23 under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

25. An article of manufacture for delivering and/or affixing a stem cell to a target tissue via juxtaposition of the article to the target tissue, comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface.

26. The article of claim 25, wherein the solid substrate is biodegradable.

27. The article of claim 25, wherein the solid substrate comprises a polymer.

28. The article of claim 25, wherein the solid substrate comprises an agent selected from the group consisting of fibrin, vicryl, hyaluronic acid, polyethylene glycol, polylactic acid, polylactic-co-glycolic acid, collagen, thrombospondin, teflon, osteopontin and fibronectin.

29. The article of claim 28, wherein the agent is teflon.

30. The article of claim 28, wherein the article is in the form of gauze, a bandage, suture, a stent, an implant or a polymeric matrix.

31. The article of claim 25, wherein the composition of matter affixed to the solid substrate further

comprises a second moiety that specifically binds to the surface of a cell in the tissue.

32. The article of claim 25, wherein the moiety is an antigen-binding portion of an antibody.

33. The article of claim 32, wherein the antigen-binding portion is a Fab fragment.

34. The article of claim 32, wherein the composition comprises a bi-specific antibody.

35. The article of claim 31, wherein the composition comprises a single polypeptide chain comprising the first and the second moieties.

36. The article of claim 25, wherein the composition comprises a recombinantly produced polypeptide chain.

37. The article of claim 31, wherein the first and second moieties are affixed via a chemical moiety.

38. The article of claim 31, wherein the first and second moieties are affixed via a polypeptide moiety.

39. The article of claim 25, wherein the stem cell is mammalian.

40. The article of claim 39, wherein the stem cell is a $CD34^+$ cell.

41. The article of claim 39, wherein the stem cell is an embryonic stem cell.

42. The article of claim 25, wherein the target tissue is selected from the group consisting of hepatic tissue, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue, skin, endothelial tissue and cardiac tissue.

43. The article of claim 42, wherein the tissue is cardiac tissue.

44. The article of claim 43, wherein the cardiac tissue is abnormal.

45. The article of claim 44, wherein the abnormal cardiac tissue is selected from the group consisting of diseased myocardial tissue, damaged myocardial tissue, diseased valve tissue, damaged valve tissue, diseased cardiovascular tissue and damaged cardiovascular tissue.

46. A method for delivering and/or affixing a stem cell to a subject's target tissue comprising contacting the tissue with the stem cell and a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

47. The method of claim 46, wherein the subject is a mammal.

48. The method of claim 47, wherein the mammal is selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rodent and a primate.

49. The method of claim 48, wherein the subject is a human.

50. The method of claim 46, wherein the contacting is performed *ex vivo*.

51. The method of claim 46, wherein the contacting is performed *in vivo*.

52. The method of claim 46, wherein the stem cell and composition of matter are first contacted with each other so as to permit the formation of a complex therebetween, and the resulting complex is contacted with the target tissue.

53. The method of claim 52, wherein the complex is contacted with the target tissue via topical administration.

54. The method of claim 52, wherein the complex is contacted with the target tissue via intravenous administration.

55. The method of claim 46, wherein the first and second moieties are antigen-binding portions of an antibody.

56. The method of claim 55, wherein the antigen-binding portions are Fab fragments.

57. The method of claim 46, wherein the composition comprises a bi-specific antibody.

58. The method of claim 46, wherein the composition comprises a single polypeptide chain comprising the first and the second moieties.

59. The method of claim 46, wherein the composition comprises a recombinantly produced polypeptide chain.

60. The method of claim 46, wherein the first and second moieties are affixed via a chemical moiety.

61. The method of claim 46, wherein the first and second moieties are affixed via a polypeptide moiety.

62. A method for delivering and/or affixing a stem cell to a subject's target tissue comprising, in no particular order, the steps of (a) juxtaposing to the tissue an article of manufacture comprising a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) contacting the article with the stem cell.

63. The method of claim 62, wherein the article is contacted with the stem cell via topical administration of the stem cell.

64. The method of claim 62, wherein the article is contacted with the stem cell via intravenous administration of the stem cell.

65. A method for delivering and/or affixing a stem cell to a subject's target tissue comprising juxtaposing to the tissue an article of manufacture comprising (a) a solid substrate having operably affixed thereto a composition of matter comprising a moiety that specifically binds to the stem cell surface, and (b) the stem cell bound to the article via the composition of matter affixed thereto.

66. The method of claim 65, wherein the solid substrate is biodegradable.

67. The method of claim 65, wherein the solid substrate comprises a polymer.

68. The method of claim 67, wherein the solid substrate comprises an agent from the group consisting of fibrin, vicryl, hyaluronic acid, polyethylene glycol, polylactic acid, polylactic-co-glycolic acid, collagen, thrombospondin, teflon, osteopontin and fibronectin.

69. The method of claim 68, wherein the agent is teflon.

70. The method of claim 65, wherein the article is in the form of gauze, a bandage, suture, a stent or a polymeric matrix.

71. The method of claim 65, wherein the composition

affixed to the solid substrate further comprises a second moiety that specifically binds to the surface of a cell in the tissue.

72. The method of claim 65, wherein the moiety is an antigen-binding portion of an antibody.

73. The method of claim 72, wherein the antigen-binding portion is a Fab fragment.

74. The method of claim 65, wherein the composition comprises a bi-specific antibody.

75. The method of claim 65, wherein the composition comprises a single polypeptide chain comprising a first and second moiety which specifically bind to the stem cell surface and tissue cell surface, respectively.

76. The method of claim 65, wherein the composition comprises a recombinantly produced polypeptide chain.

77. The method of claim 71, wherein the first and second moieties are affixed via a chemical moiety.

78. The method of claim 71, wherein the first and second moieties are affixed via a polypeptide moiety.

79. The method of claim 65, wherein the subject is a mammal.

80. The method of claim 79, wherein the mammal is

selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rodent and a primate.

81. The method of claim 80, wherein the subject is a human.

82. The method of claim 65, wherein the stem cell is mammalian.

83. The method of claim 82, wherein the stem cell is a $CD34^+$ cell.

84. The method of claim 82, wherein the stem cell is an embryonic stem cell.

85. The method of claim 65, wherein the target tissue is selected from the group consisting of hepatic tissue, epithelial tissue, connective tissue, articular tissue, bone tissue, muscle tissue, neuronal tissue, skin, endothelial tissue and cardiac tissue.

86. The method of claim 85, wherein the target tissue is cardiac tissue.

87. The method of claim 86, wherein the cardiac tissue is abnormal.

88. The method of claim 87, wherein the abnormal cardiac tissue is selected from the group consisting of diseased myocardial tissue, damaged myocardial tissue, diseased valve tissue, damaged valve tissue,

diseased cardiovascular tissue and damaged cardiovascular tissue.

89. A composition of matter comprising (a) a stem cell to be delivered to and/or affixed to a target tissue, and (b) a composition of matter comprising a first moiety that specifically binds to the stem cell surface operably affixed to a second moiety that specifically binds to the surface of a cell in the tissue.

90. A kit comprising the composition of matter of claim 1 and instructions for using same to deliver and/or affix a stem cell to a target tissue.

91. A kit comprising the article of manufacture of claim 25 and instructions for using same to deliver and/or affix a stem cell to a target tissue.

Exhibit 2

Dkt. 2259/65532-A-PCT/JPW/AJM/MVM

IN THE UNITED STATES RECEIVING OFFICE (RO/US)

Applicant : Roger Williams Hospital

International Application No.: PCT/US03/12679

International Filing Date : April 23, 2003

U.S. Serial No.: 10/553,853

For : COMPOSITIONS AND METHODS FOR STEM CELL DELIVERY

1185 Avenue of the Americas
New York, New York 10036

Attn: PCT Legal Staff
Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION IN SUPPORT OF RENEWED PETITION UNDER 37 C.F.R. §1.137(b)

I, Kimberly O'Connell, Esq., hereby declare that:

1. I am General Counsel and Vice President of Roger Williams Hospital, applicant on the above-identified PCT International Application ("subject application"), and am authorized to act on behalf of Roger Williams Hospital in connection with the subject application.

2. John P. White, Esq. of Cooper & Dunham LLP ("Cooper & Dunham"), patent counsel for Roger Williams Hospital, notified me in writing of the October 23, 2004 deadline (i.e., 30 months from the April 23, 2002 priority date) for entering the national stage in the United States for the subject application. As evidence of this written notification, I annex hereto as

Applicant: Roger Williams Hospital
Intl Appln No.: PCT/US03/12679
Intl Filing Date: April 23, 2003
Exhibit 2

Exhibits A-C copies of letters to me from Mr. White dated January 23, 2004, September 22, 2004 and October 7, 2004, respectively. The January 23, 2004 letter states, in part, that the deadline for entering the national stage in the United States is October 23, 2004. Each of the September 22, 2004 and October 7, 2004 letters states, in part, that the deadline for entering the national stage in the United States is October 30, 2004. I understood upon receipt of each of the September 22, 2004 and October 7, 2004 letters that the "October 30, 2004" date reflected a typographical error, and was intended as October 23, 2004. Each of the September 22, 2004 and October 7, 2004 letters also states that absent specific written instructions to do so, Cooper & Dunham will not enter the national stage in any country, including the United States.

3. Prior to October 23, 2004, it was my intention to enter the national stage for the subject application in the United States. However, through my oversight, I lost track of the October 23, 2004 deadline for entering the national stage in the United States and therefore did not timely instruct Cooper & Dunham to do so. Accordingly, Cooper & Dunham did not timely enter the national stage in the United States with respect to the subject application.

4. From October 23, 2004 until the present, it was never my intent not to enter the national stage in the United States.

5. Again, through my oversight, I did not act in connection with the subject application from October 23, 2004 until late June, 2005, when Alan J. Morrison, Esq. of Cooper & Dunham, after receiving a telephone inquiry in late June, 2005 from Dr. Lawrence Lum who is a coinventor named on the subject application, caused me to focus on the fact that the subject application never entered the national stage in the United States.

6. On June 27, 2005, I contacted Mr. Morrison and inquired whether any steps could still be taken to enter the national stage in the United States.

7. Between June 27, 2005 and October 5, 2005, I communicated via telephone and e-mail on several occasions with Mr. Morrison concerning the possibility of a late entry into the national stage in the United States. During these communications, Mr. Morrison discussed difficulties associated with late entry into the national stage. During these communications, I did not inform Mr. Morrison that (i) my failure to provide instructions to Cooper & Dunham by October 23, 2004 to enter the national stage in the United States was due to an oversight and was unintentional, and (ii) my not focusing on this matter prior to June 27, 2005 was likewise an oversight and unintentional.

8. During an October 5, 2005 telephone discussion with, *inter alia*, Mr. Morrison, I first informed Mr. Morrison that (i) my failure to instruct Cooper & Dunham by October 23, 2004 to enter the national stage

in the United States was due to my oversight and was unintentional, and (ii) my not focusing on this matter prior to June 27, 2005 was likewise an oversight and unintentional.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing thereon.

Dated: 2/28/06

Kimberly O'Connell, Esq.

Exhibit A

COOPER & DUNHAM LLP
ATTORNEYS AT LAW
1185 AVENUE OF THE AMERICAS, NEW YORK, NEW YORK 10036
TELEPHONE: (212) 278-0400

FILE

FACSIMILE: (212) 391-0525
(212) 391-0526
(212) 391-0630

January 23, 2004

Kimberly A. O'Connell, Esq.
Vice President and General Counsel
Roger Williams Hospital
825 Chalkstone Avenue
Providence, RI 02908

Re: PCT International Application No. PCT/US03/12679, filed April 23, 2003, on behalf of Roger Williams Hospital et al., for Compositions and Methods For Stem Cell Delivery, a continuation-in-part and claiming priority of Lawrence G. Lum et al., U.S. Provisional Application No. 60/374,929, filed April 23, 2002; Our Docket 65532-A-PCT

Dear Kim:

For your information and files, I enclose copies of the following:

1) Notification dated October 29, 2003 from the International Bureau of WIPO informing applicant that the International Application has been communicated to the designated offices other than those which have waived the requirement for such communication at this time; and

2) PCT International Publication No. WO 03/091398 A2 published on November 6, 2003 without an international search report.

Please bear in mind that the deadlines for entering the national or regional stage, in connection with this application, whichever is appropriate, under Chapter II of the Patent Cooperation Treaty are as follows:

October 23, 2004 (30 months from the earliest priority date) in: The African Intellectual Property Organization (AIPO) (Burkina Faso, Benin, Central African Republic, Congo, Cote d'Ivoire,

Applicant: Roger Williams Hospital
Intl Appln No.: PCT/US03/12679
Intl Filing Date: April 23, 2003
Exhibit A

Cameroon, Gabon, Guinea, Equatorial Guinea, Guinea-Bissau, Mali, Mauritania, Niger, Senegal, Chad, Togo), Albania, Antigua and Barbuda, Armenia, Austria, Azerbaijan, Barbados, Belize, Canada, China, Cuba, Dominica, Gambia, Germany, Ghana, Granada, Iceland, Indonesia, Israel, Japan, Kenya, the Democratic People's Republic of Korea, Republic of Korea, Lesotho, Liberia, Madagascar, Malawi, Mexico, Mongolia, Morocco, Mozambique, Nicaragua, Oman, Philippines, Poland, Portugal, Romania, Saint Lucia, Saint Vincent and the Grenadines, Seychelles, Sierra Leone, Spain, Sri Lanka, Sudan, Tajikistan, Trinidad and Tobago, Turkey, Turkmenistan, Tunisia, United Arab Emirates, United States of America, Uzbekistan, and Zimbabwe.

November 23, 2004 (31 months from priority date) in: The African Regional Industrial Property Organization (ARIPO) (Swaziland), The Eurasian Patent Organization, The European Patent Organization (Belgium, Cyprus, France, Greece, Ireland, Italy, Monaco, Netherlands, Slovenia), Algeria, Australia, Bulgaria, Belarus, Colombia, Costa Rica, Croatia, Czech Republic, Denmark (through the European Patent Office only), Ecuador, Estonia, Finland (through the European Patent Office only), Georgia, Hungary, India, Kazakhstan, Kyrgyzstan, Lithuania, Latvia, Luxembourg (through the European Patent Office only), the former Yugoslav Republic of Macedonia, Republic of Moldova, New Zealand, the Russian Federation, Slovakia, South Africa, Sweden (through the European Patent Office only), Switzerland and Liechtenstein (through the European Patent Office only), Uganda (through the African Regional Industrial Property Organization only), Ukraine, the United Kingdom, United Republic of Tanzania (through the African Regional Industrial Property Organization only), Viet Nam, and Zambia (through the African Regional Industrial Property Organization only).

February 23, 2005 (34 months from priority date) in: Bosnia and Herzegovina.

Please note that failure to enter the national or regional stage in these countries or regions by the applicable deadline will result in the abandonment of all rights to patent protections which would otherwise be available in these countries or regions based on the subject PCT Application.

Please note however, that absent specific written instructions from you to do so, we will not take steps to enter the national or regional state in any country or region except the United States unless an application having an identical content is already pending in the United States.

If you have any questions, please contact us.

Sincerely,

John P. White

JPW/MJW
Enclosures

cc: James R. McGuirk, Esq. (w/ enclosures)
Dr. Lawrence G. Lum (w/ enclosures)

Exhibit B

COOPER & DUNHAM LLP
ATTORNEYS AT LAW
1185 AVENUE OF THE AMERICAS, NEW YORK, NEW YORK 10036
TELEPHONE: (212) 278-0400

FILE

FACSIMILE: (212) 391-0525
(212) 391-0526
(212) 391-0630

September 22, 2004

* NEW YORK STATE BAR ADMISSION PENDING

BY FACSIMILE

Kimberly A. O'Connell, Esq.
Vice President and General Counsel
Roger Williams Hospital
825 Chalkstone Avenue
Providence, RI 02908

Re: PCT International Application No. PCT/US03/12679, filed April 23, 2003, on behalf of Roger Williams Hospital et al., for Compositions and Methods For Stem Cell Delivery, a continuation-in-part and claiming the benefit of U.S. Provisional Application No. 60/374,929, filed April 23, 2002; Our Docket 65532-A-PCT

Dear Kimberly:

This letter is sent to remind you that the deadline for entering the national or regional stage, whichever is appropriate, for the subject application under Chapter I of the Patent Cooperation Treaty is thirty (30) months from the U.S. priority date, i.e., **October 30, 2004**, in the African Intellectual Property Organization (OAPI) (all countries available), Albania, Armenia, Austria, Azerbaijan, Barbados, Bosnia and Herzegovina, Canada, Costa Rica, Croatia, Cuba, China, Ecuador, Equatorial Guinea, Estonia, Gambia, Guinea-Bissau, Germany, Georgia, Ghana, Granada, Iceland, Indonesia, Israel, Japan, Kenya, the Democratic People's Republic of Korea, the Republic of Korea, Liberia, Lesotho, Madagascar, Malawi, Mexico, Mongolia, Oman, Philippines, Poland, Portugal, Romania, Saint Vincent and the Grenadines, Seychelles, Sierra

Applicant: Roger Williams Hospital
Intl Appln No.: PCT/US03/12679
Intl Filing Date: April 23, 2003
Exhibit B

Leone, Spain, Sudan, Saint Lucia, Slovakia, Sri Lanka, Tajikistan, Trinidad and Tobago, Turkmenistan, Turkey, Tunisia, United Arab Emirates, the United Kingdom, Ukraine, Uzbekistan, United States of America, and Zimbabwe or thirty one (31) months from the U.S. priority date, i.e. **November 23, 2004**, in the African Regional Industrial Property Organization (ARIPO) (all countries available), the Eurasian Patent Office (all countries available), the European Patent Office (all countries available), Algeria, Australia, Bulgaria, Belarus, Colombia, Czech Republic, Denmark, Finland, Hungary, Kazakhstan, Kyrgyzstan, Lithuania, Latvia, Luxembourg, Republic of Moldova, the former Yugoslav Republic of Macedonia, New Zealand, the Russian Federation, Slovenia, Sweden, Switzerland and Liechtenstein, the United Republic of Tanzania, Republic of South Africa, Uganda, Viet Nam, and Zambia, or thirty four (34) months from the U.S. priority date, i.e. **February 23, 2005**, in Bosnia and Herzegovina.

In the light of Roger Williams' outstanding balance, and the substantial disbursements required on our part if the subject application enter the national and/or regional stage, we would require for any country or region in which the subject application is to be pursued, that the anticipated disbursements be paid in full prior to our instructing any patent attorneys to undertake work for which we would be liable.

Please provide instructions whether you wish to proceed in any country or region as soon as possible.

Please note, however, that we will not proceed to enter the national or regional stage in any country or region unless we receive specific written instructions from you to do so, and that failure to proceed by the appropriate deadline will result in Columbia's abandonment of all rights to patent protection which may otherwise be available in these countries or regions based on the subject PCT Application.

Also, I enclose for your information and files a copy of the International Search Report completed September 24, 2003 dated November 23, 2003 for International Application No. PCT/US2003/012679;

If you have any questions, please contact us.

Sincerely,

John P. White

JPW/JL
Enclosure

cc: James R. McGuirk, Esq. (w/ enclosure)
Dr. Lawrence G. Lum (w/ enclosure)

Exhibit C

COOPER & DUNHAM LLP
ATTORNEYS AT LAW
1185 AVENUE OF THE AMERICAS, NEW YORK, NEW YORK 10036
TELEPHONE: (212) 278-0400

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OF COUNSEL
DONALD S. DOWDEN

SCIENTIFIC ADVISOR
MURIEL M. LIBERTO, PH. D.

FOUNDED 1887
www.cooperdunham.com

URGENT!

October 7, 2004

BY FACSIMILE

Kimberly A. O'Connell, Esq.
Vice President and General Counsel
Roger Williams Hospital
825 Chalkstone Avenue
Providence, RI 02908

Re: PCT International Application No. PCT/US03/12679, filed April 23, 2003, on behalf of Roger Williams Hospital et al., for Compositions and Methods For Stem Cell Delivery, a continuation-in-part and claiming the benefit of U.S. Provisional Application No. 60/374,929, filed April 23, 2002; Our Docket 65532-A-PCT

Dear Kimberly:

This letter is sent to remind you that the deadline for entering the national or regional stage, whichever is appropriate, for the subject application under Chapter I of the Patent Cooperation Treaty is thirty (30) months from the U.S. priority date, i.e., **October 30, 2004**, in the African Intellectual Property Organization (OAPI) (all countries available), Albania, Armenia, Austria, Azerbaijan, Barbados, Bosnia and Herzegovina, Canada, Costa Rica, Croatia, Cuba, China, Ecuador, Equatorial Guinea, Estonia, Gambia, Guinea-Bissau, Germany, Georgia, Ghana, Granada, Iceland, Indonesia, Israel, Japan, Kenya, the Democratic People's Republic of Korea, the Republic of Korea, Liberia, Lesotho, Madagascar, Malawi, Mexico, Mongolia, Oman, Philippines, Poland, Portugal, Romania, Saint Vincent and the Grenadines, Seychelles, Sierra

Applicant: Roger Williams Hospital
Intl Appln No.: PCT/US03/12679
Intl Filing Date: April 23, 2003
Exhibit C

Leone, Spain, Sudan, Saint Lucia, Slovakia, Sri Lanka, Tajikistam, Trinidad and Tobago, Turkmenistan, Turkey, Tunisia, United Arab Emirates, the United Kingdom, Ukraine, Uzbekistan, United States of America, and Zimbabwe or thirty one (31) months from the U.S. priority date, i.e. **November 23, 2004**, in the African Regional Industrial Property Organization (ARIPO) (all countries available), the Eurasian Patent Office (all countries available), the European Patent Office (all countries available), Algeria, Australia, Bulgaria, Belarus, Colombia, Czech Republic, Denmark, Finland, Hungary, Kazakhstan, Kyrgyzstan, Lithuania, Latvia, Luxembourg, Republic of Moldova, the former Yugoslav Republic of Macedonia, New Zealand, the Russian Federation, Slovenia, Sweden, Switzerland and Liechtenstein, the United Republic of Tanzania, Republic of South Africa, Uganda, Viet Nam, and Zambia, or thirty four (34) months from the U.S. priority date, i.e. **February 23, 2005**, in Bosnia and Herzegovina.

In the light of Roger Williams' outstanding balance, and the substantial disbursements required on our part if the subject application enter the national and/or regional stage, we would require for any country or region in which the subject application is to be pursued, that the anticipated disbursements be paid in full prior to our instructing any patent attorneys to undertake work for which we would be liable.

Please provide instructions whether you wish to proceed in any country or region as soon as possible.

Please note, however, that we will not proceed to enter the national or regional stage in any country or region unless we receive specific written instructions from you to do so, and that failure to proceed by the appropriate deadline will result in Columbia's abandonment of all rights to patent protection which may otherwise be available in these countries or regions based on the subject PCT Application.

Also, I enclose for your information and file a copy of the Notification dated September 10, 2004, issued by the European Patent Office indicating that the subject PCT International Application had been assigned European Patent Application No. 03731044.8. (Please note there is no European Application actually pending).

If you have any questions, please contact us.

Sincerely,

John P. White

JPW/JL
Enclosure

cc: James R. McGuirk, Esq. (w/ enclosure)
Dr. Lawrence G. Lum (w/ enclosure)